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KRIEGSMAN & KRIEGSMAN
30 TURNPIKE ROAD, SUITE 9
SOUTHBOROUGH, MA 01772

EXAMINER	
BAUGHMAN, MOLLY E	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/544,161	BERLIN, KURT	
	Examiner	Art Unit	
	Molly E. Baughman	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-40* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-40* is/are rejected.

7)☒ Claim(s) *3,4 and 6-8* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-40 are currently pending in the application and under examination.

Claim Objections

2. Claims 3-4 and 6-8 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 3 depends from claim 2, which requires a nucleotide mixture to *only* contain 3 out of the four nucleotides, however, claim 3 introduces the fourth nucleotide, which does not further limit claim 2. Claim 6 is similar. Claims 4, and 7-8 depend from these claims.

Claim Rejections - 35 USC § 112, Second Paragraph

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 1-40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claims 1-39 are confusing because it cannot be determined what is encompassed by the term, “characterized in that.” The scope of the phrase is

unclear, and it is suggested to use conventional U.S. claim language, such as "comprising," or "wherein."

b. Claims 1, 8-9, 11-18, 23-27, and 30 are confusing because claims 1, 8-9, 11-13, 15, 23-24, and 30 do not recite any active steps. For instance, "is amplified," is not considered a positive, active step. While minute details are not required in method claims, at least the basic steps must be recited in a positive, active fashion. See Ex parte Erlich, 3 USPQ2d, p. 1011 (Bd. Pat. App. Int. 1986).

c. Claims 1-40 are confusing because it cannot be determined what is encompassed by "target DNA," and "background DNA," first cited in claim 1, and also appears in numerous dependent claims. It is unclear from the claim and specification how one can distinguish between the two types of DNA. Furthermore, not providing such description also renders the invention unclear, so it is suggested to provide further clarification.

d. Claim 1 recites the limitation "the 5-methylcytosine bases." There is insufficient antecedent basis for this limitation in the claim.

e. Claim 1 is confusing because it is unclear how amplifying the chemically treated DNA sample with the use of at least 2 primer oligonucleotides as well as a polymerase and nucleotide mixture leads to a preferred amplification of the target DNA over the background DNA. This is unclear partially because it is unclear how to distinguish between the target DNA and background DNA, as

mentioned above, and partially because the step does not provide a clear indication of how this preferred amplification occurs.

f. Claims 3 and 6 are confusing because it cannot be determined what is encompassed by "comparatively small concentration." It is unclear what such a concentration encompasses and the specification does not provide further clarification.

g. Claim 9 is confusing because the claim recites that terminating dideoxynucleotides are additionally used, however, since claim 1 only requires "a nucleotide mixture," which encompasses dideoxynucleotides, it is unclear what the terminating dideoxynucleotides are in addition to. Clarification is required.

h. Claim 10 recites the limitation "the denaturing temperature," and "the PCR amplification," in claim 1. There is insufficient antecedent basis for this limitation in the claim.

i. Claim 15 is confusing because it cannot be determined what is encompassed by the entire claim. It is unclear what exactly the other oligonucleotide or PNA oligomer is binding to, especially within the context of binding to background DNA, since it is unclear what exactly is meant by such a term. Furthermore, the description of "t" and "a" is very confusing. For instance, the claim states that "t" represents thymine at a position which correlates with an unmethylated cytosine prior to bisulfite treatment, however, this is confusing because in claim 1, the treatment converts all cytosines to uracil. It is unclear

how "'a' correlates to such a thymine position" - it is unclear what is meant by this phrase.

j. Claim 15 recites the limitation "bisulfite treatment" in claim 1. There is insufficient antecedent basis for this limitation in the claim.

k. Claim 20 recites the limitation "the other oligonucleotides" in claim 1. There is insufficient antecedent basis for this limitation in the claim.

l. Claim 25 recites the limitation "the reporter oligonucleotide" in claim 24. There is insufficient antecedent basis for this limitation in the claim.

m. Claim 26 recites the limitation "the reporter oligonucleotide or the reporter oligonucleotides" according to any one of claims 18 to 22. There is insufficient antecedent basis for these limitations in the claim.

n. Claims 37-39 provide for the use of the method according to claim 1, but, since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced. Although the claims recite the use of a method comprising particular steps, the claim does not actually provide any steps to how the method is use for the intended purpose, and therefore, the method/process applicant is intending to encompass is unclear.

o. Claim 40 is confusing because it is drawn to a nucleotide mixture according to claim 2, however, claim 2 is drawn to a method. Clarification is required.

Claim Rejections - 35 USC § 112, First Paragraph

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claim 30 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Claim 30 is drawn to concluding the presence of a disease or another medical condition of the patient from the methylation degree of the different CpG positions investigated.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention

The claims are drawn to methods of detecting cytosine methylation in a DNA sample and concluding the presence of a disease or another medical condition of the patient from the methylation degree of the different CpG positions investigated.

The breadth of the claims

The claims are broadly drawn to analyzing any DNA sample, any methylated CpG position, and then making conclusions on the presence of any disease or medical condition based on this detection.

Quantity of Experimentation

The quantity of experimentation in this area is immense since it would require collection of samples from a plethora of diseases and medical conditions, as well as control samples for each type of disease and medical condition; isolation and purification of DNA from these samples; treatment each of the DNA samples to convert unmethylated cystosine bases to uracil; amplification of such treated DNA; and making a conclusion on the methylation state of such DNA; and analyzing this in comparison to a control for each disease and medical condition in order determine whether there is a correlation of the methylation state and the presence of the disease or medical condition.

The unpredictability of the art and the state of the prior art

The art teaches that it is entirely unpredictable how the presence of any methylated CpG can be used to determine the presence of any disease. Tooke et al. (Took et al., "CpG methylation in clinical studies: utility, methods, and quality assurance," IVD Technology, Nov/Dec 2004, pg.1-5), state that in some areas of the human genome, there is normal methylation of CpG dinucleotides that controls gene expression (e.g. inactivation of the X chromosome in females). While Tooke acknowledges the association of some diseases with CpG methylation of particular genes, not all diseases or medical conditions are associated with CpG methylation. Additionally, Tooke even acknowledges that even though cancer is associated with an increase in the methylation of CpG islands, not all cancers operate in this manner (see pg.1, "Methylation and Physiology" third paragraph). Furthermore, Tooke continues on throughout the paper discussing how current technologies do not provide an absolute determination on the methylation state of CpGs in DNA. Therefore, methods of determining the presence of or diagnosing a disease or medical condition that rely on conclusions made from detected methylated CpGs in DNA are unreliable.

Working Examples

The specification has no working examples which provide a connection of any disease or medical condition with the methylation degree of different CpG positions investigated.

Guidance in the Specification.

The specification provides no guidance on how one can make a determination on a conclusion regarding the presence of a disease or another medical condition of a patient based on the methylation degree of different CpG positions investigated.

Level of Skill in the Art

The level of skill in the art is deemed to be high.

Conclusion

In the instant case, as discussed above, the level of unpredictability in the art is high regarding associating any methylated CpG with any disease, there are no working examples providing such a correlation, there is no guidance in the specification on how to make such a conclusion, and there is a high level of quantity of experimentation required. As such, one of ordinary skill in the art would be subject to undue experimentation in carrying out the claimed methods.

Claim Rejections - 35 USC § 101

7. 35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

8. Claims 2-8 are rejected under 35 U.S.C. 101 because the disclosed invention is inoperative and therefore lacks utility. Claims 2 and 5 recite the limitation of the nucleotide mixture used in the method of claim 1 only containing three out of the four

required nucleotides for amplification. It is unclear how amplification can occur without all four nucleotides present. Additionally, the specification does not provide any examples where amplification is performed using only three nucleotides.

9. Claims 37-39 are rejected under 35 U.S.C. 101 because the claimed recitation of a use, without setting forth any steps involved in the process, results in an improper definition of a process, i.e., results in a claim which is not a proper process claim under 35 U.S.C. 101. See for example *Ex parte Dunki*, 153 USPQ 678 (Bd.App. 1967) and *Clinical Products, Ltd.* v. *Brenner*, 255 F. Supp. 131, 149 USPQ 475 (D.D.C. 1966).

Claim Rejections - 35 USC § 102

10. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

11. Claims 1, 10-12, 14, 19-22, 26-27, 30-32 and 40 are rejected under 35 U.S.C. 102(b) as being anticipated by Herman et al (US 6,265,171).

Regarding claims 1, 20, and 26-27, Herman et al. teach a method for the detection of cytosine methylation in DNA samples, characterized in that the following steps are conducted:

a genomic DNA sample which comprises target DNA and background DNA is chemically treated such that all unmethylated cytosine bases are converted to uracil, while the 5- methylcytosine bases remain unchanged (see abstract and col.3);

the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, the composition of which leads to a preferred amplification of the target DNA over the background DNA (see abstract and col.3, and col.5-6, where it explains that amplification distinguishes between modified and unmodified DNA); and

the methylation state in the target DNA is concluded from the presence of an amplificate or its quantity (col.6, lines 1-6, Fig.2A-E; col.4, lines 29-48; col.9, lines 51-67).

Regarding claim 10, Herman teaches the method further characterized in that the denaturing temperature lies below 90 °C in the PCR amplification (see col.8, lines 17-18).

Regarding claim 11, Herman teaches the method further characterized in that the sample DNA is obtained from serum, plasma, urine, sputum or other body fluids of an individual (see col.7, lines 59-62).

Regarding claims 12 and 14, Herman teaches the method further characterized in that the chemical treatment is conducted with a bisulfite, disulfite, or hydrogen sulfite containing solution, and that in the chemical treatment, a reagent that denatures the DNA duplex and/or a radical scavenger is present (see col.6, lines 7-25).

Regarding claim 19, Herman teaches the method further characterized in that the polymerase used has no 5'-3' exonuclease activity (see col.9, lines 5-6, where T4 polymerase does not have 5' to 3' exonuclease activity).

Regarding claims 21-22, Herman teaches the method further characterized in that the primers in the amplification distinguish between target DNA and background DNA, and that the background DNA is methylated, while the target DNA is unmethylated, each at positions at which at least one primer for the amplification binds, whereby the one or more primers preferably bind to the target DNA after the chemical treatment (see abstract and col.6, lines 26-37).

Regarding claim 30, Herman teaches the method further characterized in that a conclusion is made on the presence of a disease or another medical condition of the patient from the methylation degree of the different CpG positions investigated (see col.21, lines 33-41).

Regarding claims 31-32, Herman teaches the method further characterized in that the amplificates themselves bear a detectable label for the detection, and wherein such labels are fluorescent labels (see col.14, lines 6-11, wherein the primers have the labels and therefor the amplificates formed from a reaction using such primers comprise the labels; and col.22, lines 57-59, same except the detectable labels are biotin).

Regarding claim 40, Herman teaches a kit consisting of a reagent containing a bisulfite, and primers for the amplification (see col. 21, lines 45-67 through col.22, lines 1-9) [It is noted that due to the indefiniteness of claim 40, "nucleotide mixture according

to claim 2," as discussed above, in this case, the claim is being interpreted as containing only the bisulfite and primers in the kit].

It is noted that due to the indefiniteness of claims 20 and 26-27, as described above, it cannot be determined how the art differs from the instant claimed invention.

12. Claims 1, 11-12, 14, and 20-27 are rejected under 35 U.S.C. 102(b) as being anticipated by Eads et al., "MethyLight: a high-throughput assay to measure DNA methylation," Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii.

Regarding claims 1, 20, and 26-27, Eads et al. teach a method for the detection of cytosine methylation in DNA samples, characterized in that the following steps are conducted:

a genomic DNA sample which comprises target DNA and background DNA is chemically treated such that all unmethylated cytosine bases are converted to uracil, while the 5- methylcytosine bases remain unchanged (see pg.ii, "Sodium bisulfite conversion and COBRA analysis");

the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, the composition of which leads to a preferred amplification of the target DNA over the background DNA (see pg.ii, "MethyLight reactions" and "MethyLight primer and probe sequences," where the primers used target either methylated or unmethylated CpG dinucleotide sites); and

the methylation state in the target DNA is concluded from the presence of an amplificate or its quantity (see abstract, and pg.ii-iii, "Quantitative RT-PCR and microsatellite instability analysis and Figure 3).

Regarding claim 11, Eads teaches the method further characterized in that the sample DNA is obtained from serum, plasma, urine, sputum or other body fluids of an individual (see abstract and pg.ii, "Sample Collection").

Regarding claims 12 and 14, Eads teaches the method further characterized in that the chemical treatment is conducted with a bisulfite, disulfite, or hydrogen sulfite containing solution, and that in the chemical treatment, a reagent that denatures the DNA duplex and/or a radical scavenger is present (see pg.ii, "Sodium bisulfite conversion and COBRA analysis").

Regarding claims 21-22, Eads teaches the method further characterized in that the primers in the amplification distinguish between target DNA and background DNA, and that the background DNA is methylated, while the target DNA is unmethylated, each at positions at which at least one primer for the amplification binds, whereby the one or more primers preferably bind to the target DNA after the chemical treatment (see pg.ii, "MethyLight reactions" and "MethyLight primer and probe sequences," where the primers used target either methylated or unmethylated CpG dinucleotide sites; and Figure 3).

Regarding claim 23-27, Eads teaches the method further characterized in that additionally at least one reporter oligonucleotide is used in the amplification whose fluorescence properties change as a consequence of the amplification [claim 23]; wherein a Taqman assay or a LightCycler assay or an assay with the use of Molecular Beacons is conducted to conclude upon the methylation state at the last step of the method [claim 24]; that the reporter oligonucleotide bears at least one fluorescent label

[claim 25] (see abstract, and pg.ii, "MethyLight reactions" and "MethyLight primer and probe sequences").

13. Claim 40 is rejected under 35 U.S.C. 102(b) as being anticipated by Das et al. (US 6,143,504).

Das teach a kit comprising bisulfite, primers and a nucleotide mixture (see col.4, lines 24-34).

14. Claim 40 is rejected under 35 U.S.C. 102(b) as being anticipated by Cottrell (US 6,960,436).

Cottrell teach a kit comprising bisulfite, primers and a nucleotide mixture (see col.7, lines 1-5; and col.10, lines 28-55).

Claim Rejections - 35 USC § 103

15. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

16. Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Herman et al (US 6,265,171) or Eads et al., "MethyLight: a high-throughput assay to measure DNA methylation," Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii,

in view of Yuanxiang et al., "Use of a Single Sequencing Termination Reaction to Distinguish Between Cytosine and 5-Methylcytosine in Bisulfite-Modified DNA," Biotechniques, May 1997, Vol.22, pp.850-853.

The teachings of Herman et al. and Eads et al. are discussed above. Although these references discuss the amplification reactions comprising sequencing (see col. 22, lines 37-67 of Herman, and pg.iii, "Bisulfite genomic sequencing"), they do not particularly state that such reactions include terminating dideoxynucleotides.

Yuanxiang et al. discuss a method of analyzing methylation in DNA by a reaction which distinguishes between cytosine and 5-Methylcytosine in bisulfite-modified DNA (see title). Such a reaction comprises using terminating dideoxynucleotides during the reaction (see Figure 1 and pg.851, right column and pg.853, last paragraph).

One of ordinary skill in the art would have been motivated to modify the method of Herman et al. or Eads to include terminating dideoxynucleotides in the amplification because Yuanxiang demonstrates that it was conventional in the art at the time of the invention to include terminating dideoxynucleotides into amplification reaction for the benefit of sequencing the DNA when analyzing methylation. The skilled artisan would have had a reasonable expectation of success in further including terminating dideoxynucleotides in the amplification of Herman et al. or Eads et al. for the added benefit of sequencing the DNA for determining the methylation state. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed terminating dideoxynucleotides therein.

17. Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Herman et al (US 6,265,171) or Eads et al., "MethyLight: a high-throughput assay to measure DNA methylation," Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii, in view of Guetig (US 2004/0248120).

The teachings of Herman et al. and Eads et al. are discussed above. Neither Herman nor Eads discuss the method where the chemical treatment is conducted after embedding the DNA in agarose.

However, Guetig explains that treating DNA with bisulfite after it is embedded in agarose was a conventional practice in the art at the time of the invention (see paragraph [0017]). Guetig explains that such a method allows for the prevention of the diffusion and renaturation of DNA to occur during the treatment process, thereby allowing the DNA to stay single-stranded, which is required for bisulfite treatment. Therefore, the skilled artisan would have had a reasonable expectation of success in embedding the DNA in agarose when chemically treating the DNA in the method of Herman et al. or Eads et al. since Guetig explains that such a practice was conventional in the art at the time of the invention. It would have been *prima facie* obvious to one of skill in the art at the time of the invention to carry out the claimed methods and include the claimed DNA embedded in agarose therein.

18. Claims 15-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over either one of Herman et al (US 6,265,171) or Eads et al., "MethyLight: a high-throughput assay to measure DNA methylation," Nucleic Acids Research, 2000, Vol.28, No.8, e32,

pgs.i-viii, in view of Orum et al., "Single base pair mutation analysis by PNA directed PCR clamping," Nuc. Acids Res., 1993, Vol.21, No.23, pp.5332-5336.

The teachings of Herman et al. and Eads et al. are discussed above. Herman and Eads do not discuss the method where the amplification is conducted in the presence of at least one other oligonucleotide or a PNA oligomer as recited in claim 15, 16, 17, or 18.

Orum et al teaches a method of PCR clamping in which PCR amplification is inhibited by the use of primers that cannot be extended. Orum explains how the PNA will compete with the PCR primer for any primer sites in the genome, thereby suppressing any occurence of non-specific background in the PCR process directed by this primer (see pg.5336, second paragraph). Orum also discusses how the method can be applied in reverse manner, where the PNA clamps target non-mutated genes, thereby leading to reduction of unwanted background amplification (see pg.5336, last paragraph). Although Orum uses the method to detect mutations, the principle of the method uses such PNA oligomers in the detection of particular targeted nucleotide bases in the genome, and one of skill in the art could reasonably conclude that the method could also be used to analyze targeted cytosines, as in the method of Herman et al. or Eads et al. Therefore, it would have been obvious for one of ordinary skill in the art at the time the invention was made to modify the method of Herman et al. or Eads et al. to utilize further oligonucleotides or PNA oligomers that impede the binding of primer oligonucleotides to background DNA because Orum demonstrates the benefits of utilizing PNA oligomers in single base detection amplification reactions in

order to reduce background amplification and increase signal strength. The skilled artisan would have had a reasonable expectation of success in utilizing the PNA clamp principle for single base detection of Orum in the method of Herman et al. or Eads et al. in order to reduce background amplification and increase signal strength of target DNA when analyzing the methylation state. It would have been *prima facie* obvious to one of skill in the art at the time of the invention to carry out the claimed methods and use the claimed further oligonucleotide(s) or PNA oligomer(s) therein.

19. Claims 28 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Eads et al., "MethyLight: a high-throughput assay to measure DNA methylation," Nucleic Acids Research, 2000, Vol.28, No.8, e32, pg.i-viii.

The teachings of Eads et al. are discussed above. Although Eads does not specifically discuss the method wherein the background DNA is present in 100X or 1000X concentration in comparison to target DNA, he does discuss conducting a similar assay where fully methylated human sperm DNA (by treatment) is serially diluted in 10-fold increments up to 1:100,000 with untreated, unmethylated human sperm DNA in order to test the sensitivity and quantitative accuracy of the MethyLight technique (see Figure 4). Eads explains that such an assay exemplifies situations where one would want to detect aberrant methylation patterns in human samples with substantial contamination of normal DNA, such as non-microdissected, heterogeneous tissue samples (see pg.v, "Sensitivity and quantitative accuracy of MethyLight technology"). Therefore, although in the Eads method both the types of DNA are not chemically

treated, one of skill in the art could reason that his example achieves the same predictable result of being able to detect methylation patterns in samples which are highly contaminated with non-target DNA up to 1000X.

20. Claims 28-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Herman et al (US 6,265,171) in view of Eads et al., "MethyLight: a high-throughput assay to measure DNA methylation," Nucleic Acids Research, 2000, Vol.28, No.8, e32, pgs.i-viii.

The teachings of both Herman and Eads are discussed above. Herman does not discuss the method wherein the background DNA is present in 100X or 1000X concentration in comparison to target DNA.

Eads discusses conducting a similar assay where fully methylated Human sperm DNA (by treatment) is serially diluted in 10-fold increments up to 1:100,000 with untreated, unmethylated human sperm DNA in order to test the sensitivity and quantitative accuracy of the MethyLight technique (see Figure 4). Eads explains that such an assay exemplifies situations where one would want to detect aberrant methylation patterns in human samples with substantial contamination of normal DNA, such as non-microdissected, heterogeneous tissue samples (see pg.v, "Sensitivity and quantitative accuracy of MethyLight technology"). Therefore, although in Eads method both the types of DNA are not chemically treated, one of skill in the art could reason that his example achieves the same predictable result of being able to detect methylation patterns in samples which are highly contaminated with non-target DNA up to 1000X.

One of skill in the art would have been motivated to modify the method of Herman et al. to conduct the assay where the background DNA is present in 100X or 1000X concentration in comparison to target DNA because Eads demonstrates that such an assay exemplifies situations where one would want to detect aberrant methylation patterns in human samples with substantial contamination of normal DNA, such as non-microdissected, heterogeneous tissue samples. Therefore, the skilled artisan would have had a reasonable expectation of success in conducting the method of Herman et al. using background DNA at 100X or 1000X concentrations in comparison to target DNA for the benefit of conducting the assay in a manner that mimicked one using non-microdissected, heterogeneous tissue samples in order to ensure sensitivity and high quantitative accuracy in the method. It would have been *prima facie* obvious to one of skill in the art at the time of the invention to carry out the claimed methods and include the claimed sample ratios therein.

21. Claims 33-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Herman et al (US 6,265,171) in view of Olek et al. (WO 01/77378 A2, published October 18, 2001).

The teachings of Herman et al. are discussed above. Although Herman discusses the method where the amplificates themselves bear a detectable label for detection, he does not discuss the labels being radionuclides, or where they are removable mass labels which are detected in a mass spectrometer. Although he discusses primers comprising biotin groups, where the amplificates can then be bound

to streptavidin coated beads following amplification (col.22, last paragraph), he doesn't particularly discuss the reaction occurring on the beads.

Olek et al. discuss methods of detecting cytosine methylation state in DNA where primers are attached to a solid phase during amplification (see pg.9 and 11-12). Olek also discusses the amplificates bearing labels including radionuclides, or detachable mass labels which can be detected in a mass spectrometer (see pg. 12, second paragraph).

One of ordinary skill in the art would have been motivated to modify the method of Herman et al. to use radionuclides as labels or removable mass labels, or conduct the method with the primers bound to a solid phase because such techniques were conventional in the art at the time of the invention in analyzing cytosine methylation patterns in DNA, as demonstrated by Olek et al. Since Herman demonstrates the benefits of using labels to detect amplificates, as well as using solid phases in the isolation and detection of amplificates, and Olek demonstrates that it was conventional in the art at the time of the invention to use removable mass labels which can be detected in a mass spectrometer, radionuclide labels, and to also conduct amplification where the primers are bound to a solid phase, it would have been obvious to one skilled in the art to substitute label and detection technique for the other to achieve the predictable result of detecting cytosine methylation patterns in DNA labeled various types of labels.

Double Patenting

22. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

23. Claims 1 and 8-36 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-36 of U.S. Patent No. 7, 229, 759. Although the conflicting claims are not identical, they are not patentably distinct from each other because of a genus:species relationship. For instance, the instant method of claim 1 is identical to that of claim 1 of the '759 patent with the exception of the blocking oligonucleotide or PNA oligomer. However, the blocking oligonucleotide or PNA oligomer is claimed in dependent claims 15-18.

Summary

24. No claims are free of the prior art.

25. Radlińska et al., "Novel procedure for the detection of 5-methylcytosine," Acta Microbiologica Polonica, 1998, Vol.47, No.4, pp.327-334, is noted as a reference of interest. The article discusses conducting primer extension reactions (not amplification) using only three nucleotides to detect methylated cytosines.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Molly E. Baughman whose telephone number is (571)272-4434. The examiner can normally be reached on Monday-Friday 8-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Kenneth R Horlick/
Primary Examiner, Art Unit 1637

/Molly E Baughman/
Examiner, Art Unit 1637